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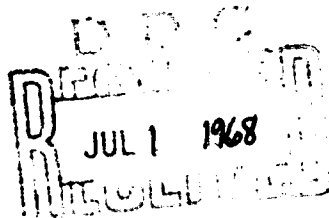
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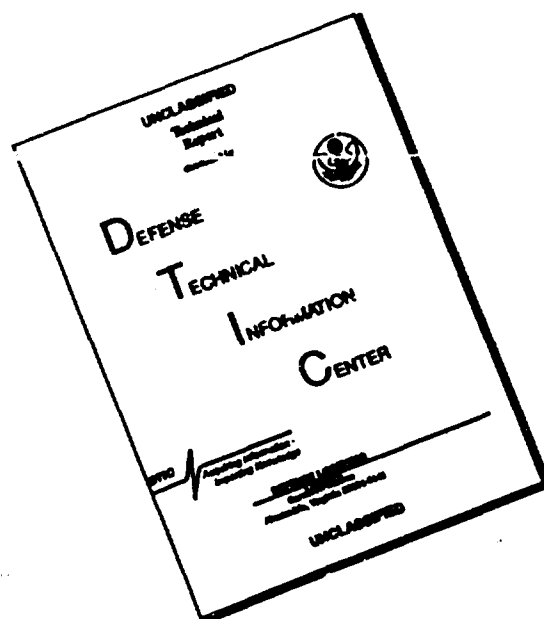
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INTERMEDIATE REPORT FOR RESEARCH PROJECT 71-310-L-307, 1966

"RETENTION PROPERTIES OF MEMBRANE FILTERS AND OTHER FILTERS
FOR VIRUSES FROM AIR"

(Following is a report by Prof. Dr. H. Brandis, Director
Hygiene Institute of University of Gottingen, Germany.
Translation performed by Constance L. Lust.)

It was to be assumed that membrane filters and other similar filters
have a retention property for filtering viruses from air. This property
is not absolute. It was our task in the research project to obtain
quantitative data about high capacity of filtering from virus-aerosols.
Also quantitative data were needed about the degree of (presumeably
small) penetrability of the filters. The most sensitive tests were
to be used to show this.

Our course had to consider the following points:
1) Determining the filter capacity, 2) Possibilities for a sensitive
demonstration of virus aerosols, 3) Retention properties of filters for
viruses in aerosols.

In order to find a suitable experimental arrangement (nozzle, course
of aerosol, chamber, etc.) we undertook a literature study at first.
At the same time this was to inform us, whether other similar investigations
were already in progress.

1) Literature Study:

We could not find any reports with quantitative data about the re-
tention properties of filters for filtering virus aerosols.

Spray nozzle: In the U.S.A. the "Collison--Spray" is used extensively,
which makes 80-85% of the droplets between 3.0 and 0.5 microns. It is
also built into the "Henderson Aerosol Apparatus", as was described by
Henderson in 1951. Other apparatuses to make smoke (fog) found in the
american literature are described by Borgman et al. (1961). In the
russian literature "Burkow's duster" is mentioned frequently, as for
example by Gaidamovitsch and Vlodavec (1963). For us it was easiest
to obtain the nozzle (Neusilber Nozzle) from Drager Lubeck (M218), where
particles are 13.7% under 0.7 micron, 85.7-89.9% between 0.7 and 5 microns.
Only a very small percentage of particles are greater than 5 microns.
This nozzle was similar to the "Collison-Spray" and was suitable for our
experiments.

Apparatus for releasing and containing aerosols:

In the literature two principles are described:
a) dynamic system = aerosol is in contrast circular motion and new

aerosel replaces the used or released aerosol.

b) static system = aerosol is in large chambers and by removing samples its stability, sedimentation etc, is studied.

The dynamic system is incorporated in the Henderson-apparatus, which is well suited for studying infections via aerosols (Elberg and Goldberg 1947; Dolfe 1961). Because of the difficulty in obtaining it in the time allotted to this project, as well as because of its $6,000 price tag it was not considered for these planned experiments. A disadvantage of this equipment appeared to be the impossibility of sterilization; the procedures for disinfecting the apparatus appeared to be problematical to me.

Aerosol chambers were described by Elberg and Henderson (1947), Harper et al. (1967), Hood (1963) and Ehrlich et al. (1964). A thorough overall view about the various types is described by Wolfe (1961); several good descriptions also by Goldberg et al. (1958) and Zhahlko-Titarenko (1960). The chambers are in part rotating drums and are rather large (up to 1600 liter). An aerosol chamber in which a volunteer subject can be exposed to aerosols was described by Griffith (1963). These chambers appeared to be not wholly suited for our experiments because of unintential loss and mixing of the aerosol.

Methods for demonstrating virus in aerosols:

Bacteria have been shown to be in aerosols for many years (Wills 1933, Moulten et al. 1943). Various techniques are used in these methods. Of particular interest is the "Anderson-Sampler" which also gives separation of particle size (Anderson 1958). Two principles are found in the literature for demonstrating viruses in aerosols. a) Collecting the aerosol in liquids, or via adsorption-aerosols, b) collecting the aerosols on filters.

Collection of aerosols in liquids is carried out with impingers of various kinds and was described comprehensively by Tyler and Chipe (1955) and in the Public Health Monograph No. 686 (1959). In the english literature the "All-Glass-Impinger" is used extensively, in the russian literature the "Djakonow-Apparatus" (Vlodavec, 1957, 1958; Gaidamovitsch et al. 1959) is used primarily. Collecting aerosols by adsorption on a second aerosol is the principle used in the collector of Recomensky described in the russian literature (also Gaidamovitsch 1963), as well as in the "atomizer-sample" of Moulton (Albrecht 1955). Gelatin filters are mentioned primarily as filters for viruses.

Several leads as to the performance of various methods are found in the Public Health Monograph Publication No. 686 (1959), and Publication No. 953 (1962), and in Vlodavec (1957, 1958). We found no quantitative results about collecting viruses from air.

2) Testing the materials:

In order to avoid experimental errors because of residual virus,

or if this was avoided by disinfectants, then the chemicals, we chose to utilize a demountable apparatus the individual components of which could be sterilized by autoclaving. The vessels were of glass, connectors PVC tubing, the nozzle of nickel silver (German silver), the filter holding "clamp" of metal. The filter was of synthetic materials and could be used only once.

We began the experiments with a bacteriophage. Coliphage T_3 was suitable, it made large phages as an indicator strain E. coli B. This was especially useful, since we wanted to demonstrate bacteriophages on the filter by means of bacterial strain. The bacterial growth was to develop on these filters.

From the large number of impingers we chose the Djakonow-Apparatus mentioned in the russian literature (this was made by glass blowers according to descriptions in the literature), as well as the All-Glass-Impinger mentioned in the english literature (All Glass Inc, Vineland, N. J.). We added 1-2 drops of defoamer E 100 (Silicon emulsion Farbenfabriken Bayer, Leverkusen) We used 5 ml. Bacto Nutrient broth for the Djakonow apparatus, and 20 ml for the All-Glass-Impinger. Antifoamer was added after we had satisfied ourselves that it had no inactivating effect on phages. We could not use the alginate filters because of their extremely low ability to pass air. We used 3 kinds of filters: 1) gelatin filters (MF gelatine) (50 mm) from the Membrane Filter Co. of Gottingen, 2) membrane filter MF 10, 12, 15 (50 mm) same company as in (1) and VP 10 (47 mm) HA V.45 (37 mm) from Millipore Filter Corp., Bedford Mass., 3) Microsorbane filters (50 mm) from Delbag Air Filter Company, Berlin. As filter supports we used those of the Membrane Filter Corp and the Clinical Monitors of synthetic material which come with the Millipore HA V.45 filters. As we observed the ability of filters to pass air, we concluded that perhaps the aerosol passes through the filter in the support area. We therefore also used (later) a high pressure filter holder (Millipore Corp.), which circumvented this danger in large part.

3) Testing of the conditions:

From the literature it was seen that the best physical conditions for aerosols are present when one works in the dynamic system (flowing). We therefore wanted to duplicate these conditions in our trials. Thus far this has not been wholly successful; technical difficulties arose in that the pressure and suction made an simultaneous aerosol channel which could only be directed to the indicator system after all conditions and equilibrated (stablized).

Initially we started preliminary experiments with a mixed dynamic static system with which we did not have these difficulties (Figure 1). The aerosol was made under constant pressure, which came from a compressed air tank via a reduction valve. The aerosol was carried via connecting tubing to a chamber (2+6 liter glass jars) and there it introduced through a flow meter

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to 2 or 3 impingers (Fischer and Porter Corp., Gottingen). The chamber thusly filled with aerosol. After a "spray time" of 4 or 8 minutes this part of the trial was stopped. The take up and release valves (Solidex 5 mm) of the aerosol chamber were closed and the aerosol was sucked over 2 impingers or 2 filters via a third valve with a pump in the second part of the experiment. The physical disadvantages of this method lie in the fact that high density of aerosol in the first part and in the lowering air pressure in the second. The latter effect may alter the vapor pressure of the aerosol and consequently perhaps change the particle size. We thought we could begin the experiments because the air temperature was 22^0-24^0C and relative humidity of the room was 25%-45%. An advantage of the method was that the transient static conditions of the aerosol in the chamber aid in establishing equilibrium with the course dispersion (the chambers were 2 or 6 liters). Two or six liters of aerosol could be siphoned off and the results could be compared on a basis of per liter of aerosol.

It became apparent that a constant diameter of aerosol were desirable in the system, and no sharp deviations were allowable. In all places where aerosol came in contact with objects a settling occurred. We kept the width of 5 mm in the glass tubing and 7 mm in the PVC tubing. This aspect of the trial virus was checked with the aid of "colored-aerosols."

Methods

1) Impinger experiments (see also Fig. 1)

The compressed air is introduced at a constant 1.5 atm via a PVC tubing into the nozzle. The nozzle is placed into the glass vessel so that the level of liquid is below the nozzle. The liquid to be aerosolized is sucked up thru a short piece of tubing. In this way the larger aerosol droplets fly against the wall of the container and flow back into the phage suspensions. Only the finer droplets of loaded aerosol reach the aerosol chamber.

At the beginning of the experiment valves I and II of the chamber are opened. The source comes via tap I, thru II the take off over impinger I and II. A flow meter is attached after the impingers. The "spray time" is kept constant at 4 or 8 minutes in order to standardize the method. The nozzle delivers 6 liters of aerosol per minute under those conditions.

After stopping this part of the experiment the air pressure is curtailed by closing valve I and II. Tap III is opened and the 2 liters (or 6) inside the chamber are sucked up over impingers III and IV. The number of phages so collected on the impingers is a measure of the concentration of the phage in the aerosol chamber.

In this experiment the quantity of phage-suspension in the 4 or 8 minute spray were also determined gravimetrically. An average valve was 0.6-1.2 ml. If this volume is converted to the concentration in

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the original suspension one obtains the number of "sprayed phages", and this can be compared to those collected by the impingers. The difference between the sprayed and collected number of phages is due to physical and biological loss.

2) Filter experiments

The procedure was similar to that of the impinger experiments. Instead of impinger I and II a hose is connected directly to a flow meter, and instead of impinger III and IV tap III is connected to a filter holder. The experiment is again done in two parts. The chamber is filled; the contents are then sucked over a filter. One must differentiate between a) use of soluble filters which can give quantitative data similar to the impingers, b) use of water-insoluble filter which only yield quantitative results with low phage concentrations. At high concentrations one can only utilize their retention properties.

Group a) Gelatin filters. They allow air to pass (2.7 liters per min. cm^2 at a pressure differential of 500 mm WS; according to the supplier) and are water soluble, especially when the solution is warmed somewhat.

They are weak and tear, and only few experiments succeeded where the filters did not break. Gelatin filters are available "fine" and "coarse". This supposedly (supplier) corresponds to a porosity of; coarse 2.0 microns Hagen-Poiseuille and 3.0 microns by mercury intrusion phenomenon; fine 0.6 microns and 0.45 microns respectively.

We used coarse filters mainly, since the fine filters were technically difficult to make. The use of the gelatin filters was similar to that of impingers. Right after use the gelatin filters were dissolved in 5.0 ml Bacto Nutr. Broth at 37oC. Solution is complete after 20 minutes. The solution was subsequently titrated and worked up.

Group b) Membrane filters MF10, 12, 15, VF 10, HA 0.45. According to the supplier these filters hold back materials on their upper surface.

The microsorbane filters are also water-soluble, but are not made according to the principle of the membrane filter. They filter by a deep-filtration action.

The supplier lists the pore sizes as: MF 10 by Hagen-Poiseuille 0.1 microns; by Hg intrusion 0.01 micron; MF 12 0.15 + 0.05, MF15 0.27 + 0.15; VF 10 10 ± 2 micron, HA 0.45 0.45 microns (± .2) No pore size was listed for microsorbane filters.

These filters were used as follows (except microsorbane); right after the trial the filters were placed, bottom side down, on a dried agar plate and overlayed with enough Coli-Bouillon (overnight culture, 2 hr. culture). The filter is then dried, with the lid open, for 20 minutes in an incubator. Next morning the counting of phages and other calculations are made.

5.

The first filter can only be used at low phage concentration, since at 10^4/ml confluent lysis is observed. The retention properties of the first filter are checked with the aid of a second filter in the row.

Microsorbans filters could not be used with the agar plate method because of their special construction. With those filters the retention properties (capacity) were merely investigated.

Results

1) Dose of sprayed bacteriophages:

Two sets of experiments were employed so that we would not have to measure the dose of phage to be sprayed. The nozzle was used at constant air pressure for 4 and 8 minutes. The sprayed volume of bacteriophage suspension was therefore determined gravimetrically. The nozzle and tubing were simply weighed before (empty) and when full with phage suspension. We found that a spray time of 4 minutes used an average volume of 0.6 ml to make spray; for 8 minutes the average "sprayed-volume" was 1.2 ml (see table 1).

Therefore by keeping the conditions constant we could assume that in our further experiments the dose of sprayed bacteriophage was constant.

2) Recovery of bacteriophages:

Of the sprayed phages an average of 25% were recovered in the impingers. The greatest quantity was found in impinger I; impinger II only received 17% of that of I; impinger IIa about 44% of II (see table 2, 3a and 3b). There was no apparent difference between the Djakonow-Apparatus and the All-Glass-Impinger. The phages recovered from the chamber were only a small percentage of the total recovered; at the 4 minute spray time in a 2 liter chamber was about 0.85% of the sprayed phages (see table 2). In impinger IV about 42% of impinger III was still found (see table 2, 3a, 3b). The relationship between the dose of sprayed phages and the total recovered was relatively constant.

3) Number of phages in the chamber aerosol:

It was important to determine the number of phages in the aerosol in the chamber in reference to the experiments with water-soluble filters since this was important to calculate the filter capacity. As already mentioned (2) the number of phages recovered on impinger III and IV and in the chamber were in a relatively constant relationship with the amount of sprayed phages, when the spray-time and the volume of the chamber were constant; 0.85% of the sprayed phages were then recovered in the chamber. When the spray-time was doubled and a 6 liter chamber this relationship must change according to the ratio 3:2. This was the case. The phages recovered from a 6 liter chamber with an 8 minute spray time were 1.3% of the sprayed phages (Table 3a and 3b). In this case the Djakonow and All-Glass-Impinger gave the same values. The results obtained with impingers were compared with those of gelatin filters. If the same results were obtained by using two different starting principles the conclusions can be assessed with greater certainty.

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Table 4 shows that with an 8 minute spray time with gelatin filter 1.5% of the sprayed phages were recovered in the 6 liter chamber.

Starting with different concentrations provided additional proof that a constant ratio existed between the number of "sprayed phages" with respect to a definite phage-concentration in the aerosol chamber. In figure 2 it can be seen that a linear relationship exists for impingers as well as for gelatin filters between the starting concentration and phage concentration in the aerosol chamber. The slope of the relationships illustrated was about 45°.

Since impingers and gelatin filters yield equivalent results about the concentrations in the aerosol chamber, these results appear to represent the actual phage concentrations in the chamber. It represents a useful statement (specification) about the extent of the capacity of the filter through which the contents of the aerosol chamber are conducted.

c) The sensitivity of different methods for demonstrating bacteriophages from aerosols:

In figure 2 one can also see the relationship of the phages remaining in the chamber to the dose of the sprayed phages in the different methods. At higher concentrations of starting concentrations the gelatin filters show somewhat more phages in the chamber than the third impinger; at low concentrations the reverse was true. The deviations are too small to be able to draw further conclusions. The relationships would be different if the "further-removed" impingers were included and their phage count added in. Water insoluble membrane filters -described earlier- are less sensitive in demonstrating phages in aerosols than are impingers and gelatin filters. Because of the simplicity of evalution (placing the filters on agar plates and reading plaques directly) they are very well suited at low aerosol concentrations. They are also suited to use as a control for a few phages.

In summary: The present results showed that the instrumentation and methods are capable of yielding useful and reproducible results. We were able to start experiments about retention properties of different filters. The results are not complete as yet. This will be referred to in a subsequent report.

H. Brandis

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Figure 1

ABBILDUNG I. VERSUCHSANORDNUNG

AEROSOL-KAMMER
Chamber

DÜSE
Nozzle

DURCHFLUSS-MESSER
flow meter

AUSTRITT

IMPINGER

PRESSLUFT
Compressed air

PUMPE
pump
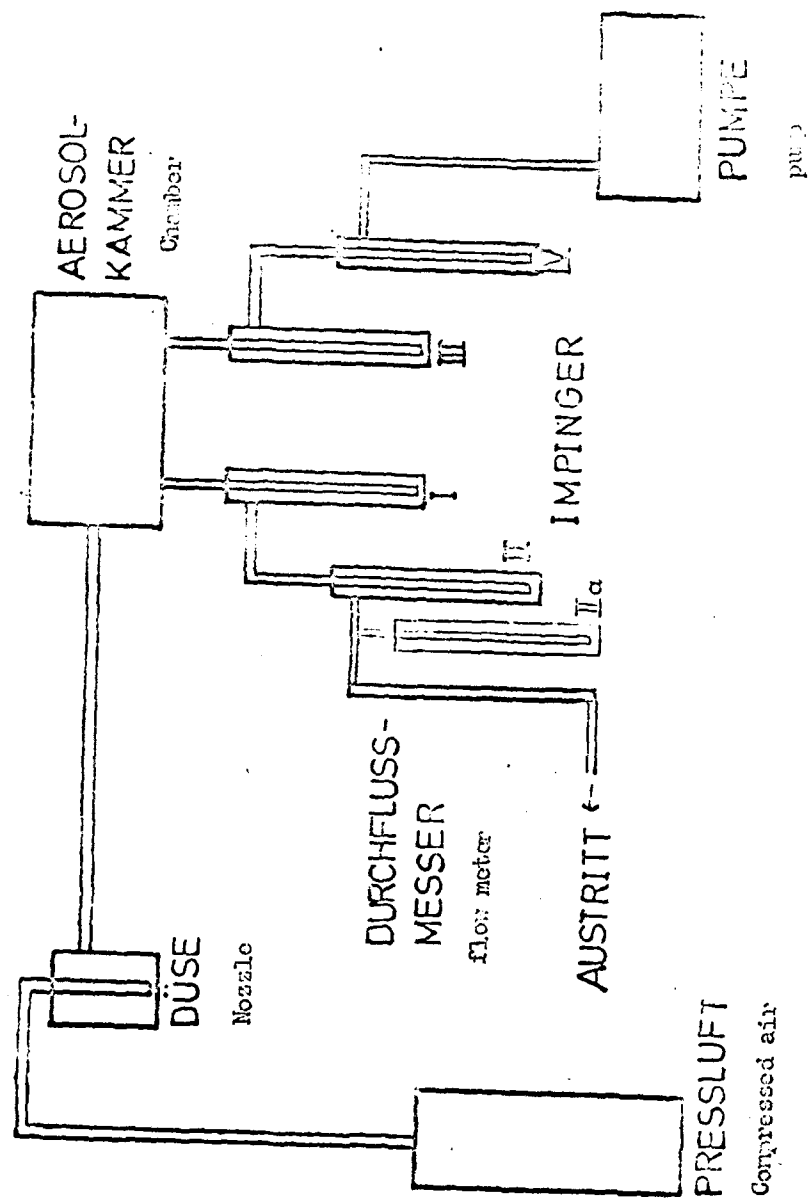
Tabelle 1

Gravimetrische Bestimmung der versprayten Phagen-
suspensionen in ml

Gravimetric Determination of sprayed phages (in ml.)

Trial	Sprayzeit Spray time	
	4 min	8 min
Versuch Nr.1	0,55	1,30
" " 2	0,60	1,55
" " 3	0,65	1,20
" " 4	0,64	1,20
" " 5	0,65	1,20
" " 6	0,61	1,10
" " 7	0,54	1,15
" " 8	0,55	1,10
" " 9	0,65	1,10
" " 10	nicht durchgeführt	1,25
Mittelwert der Versuche 1-10	0,60	1,21

Average of
trials

9.

Tabelle 2

Results with Djakonow Apparatus and chamber of 2 liters.

Ergebnisse mit Djakonow-Apparaten und einer Aerosolkammer von 2 Liter Inhalt.

phage titer/ml. Phagensusp.- Titer / ml	Spray-dose Versprayte Phagendosis	Djakonow-Apparate I	II	IIa	III	IV	spray-dose Versprayzeit V = Versprayzeit A = Absaugzeit
$6,0 \times 10^6$	$3,3 \times 10^6$	$5,3 \times 10^5$	10×10^4	$3,5 \times 10^4$	$3,5 \times 10^4$	nicht bestimmt	V = 4 min. A = 1 1/2 min.
$6,0 \times 10^6$	$3,6 \times 10^6$	$8,0 \times 10^5$	$7,0 \times 10^4$	$3,2 \times 10^4$	$2,6 \times 10^4$	$1,3 \times 10^4$	V = 4 min. A = 4 min.
$7,1 \times 10^6$	$4,6 \times 10^6$	11×10^5	$6,0 \times 10^4$	$1,9 \times 10^4$	$2,5 \times 10^4$	$0,4 \times 10^4$	V = 4 min. A = 4 min.
$9,2 \times 10^6$	nicht bestimmt *	10×10^5	$9,0 \times 10^4$	$3,0 \times 10^4$	nicht bestimmt	nicht bestimmt	V = 4 min. A = 4 min.
$8,1 \times 10^8$	$5,3 \times 10^8$	$1,3 \times 10^8$	$0,4 \times 10^7$	$2,3 \times 10^6$	$5,8 \times 10^6$	$1,7 \times 10^6$	V = 4 min. A = 4 min.
$8,2 \times 10^8$	$5,0 \times 10^8$	$1,4 \times 10^8$	$2,4 \times 10^7$	$9,0 \times 10^6$	$5,0 \times 10^6$	$2,1 \times 10^6$	V = 4 min. A = 4 min.
$8,2 \times 10^8$	nicht bestimmt	$1,3 \times 10^8$	$0,4 \times 10^7$	$5,8 \times 10^6$	$1,7 \times 10^6$	nicht bestimmt	V = 4 min. A = 4 min.
$8,2 \times 10^8$	nicht bestimmt	$1,6 \times 10^8$	$6,5 \times 10^6$	nicht bestimmt	nicht bestimmt		V = 4 min. A = 4 min.
$3,2 \times 10^8$	nicht bestimmt	$1,2 \times 10^8$	$9,0 \times 10^6$	nicht bestimmt	nicht bestimmt		V = 4 min. A = 4 min.
$9,3 \times 10^8$	$5,2 \times 10^8$	$1,3 \times 10^8$	$0,6 \times 10^7$	$3,2 \times 10^6$	$4,3 \times 10^6$	$3,2 \times 10^6$	V = 4 min. A = 4 min.

* not done

Tabelle 3 a

Results with Djakonow Apparatus and a chamber of 6 liters
Ergebnisse mit Djakonow-Apparaten u.einer Aerosolkammer von 6 Liter Inhalt
(Versprayzeit 8 min.) Spray-time 8 minutes

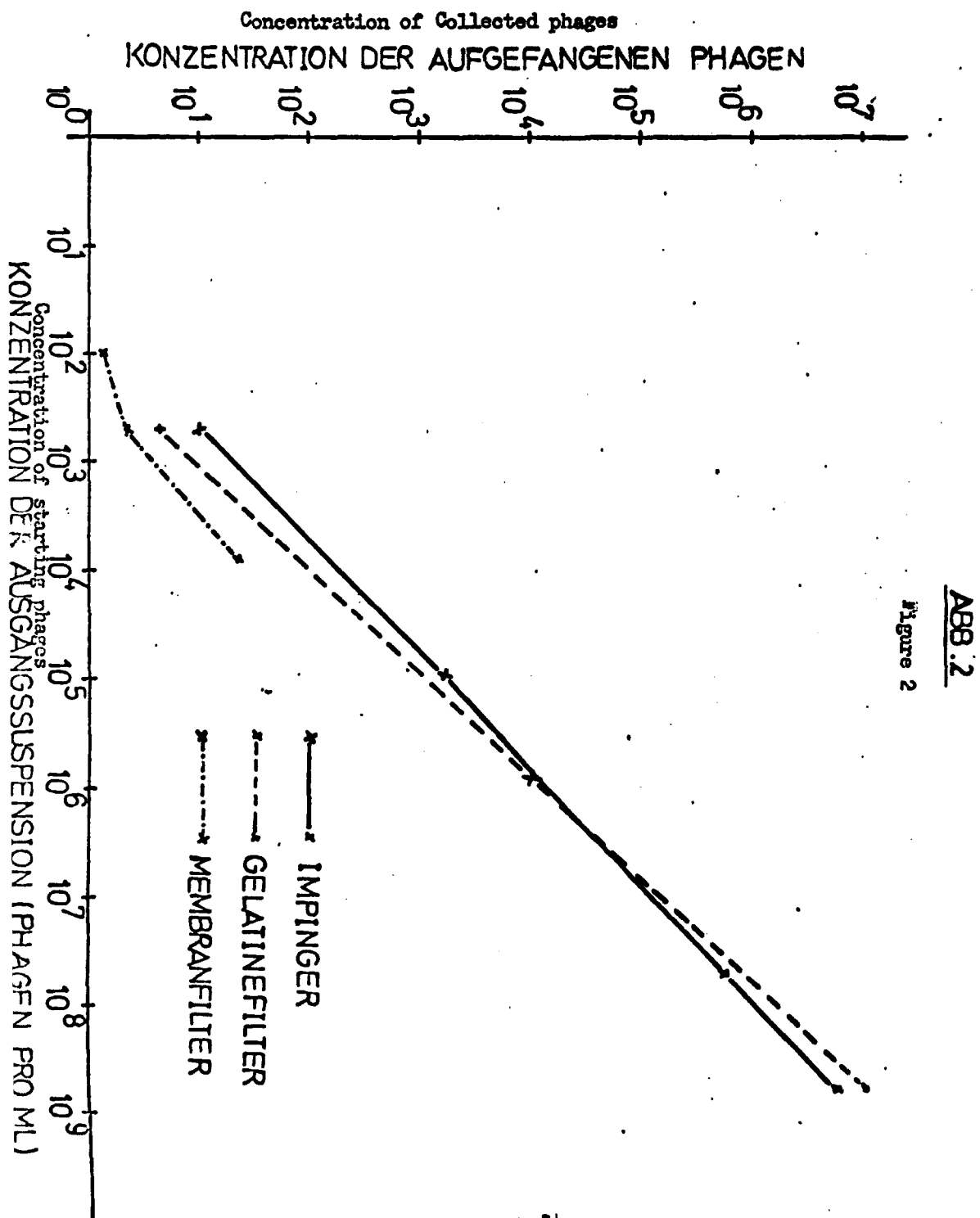| Phagensusp. Liter / ml	Versprayte Phagendosis	Djakonow-Apparate I	II	III	IV
$5,6 \times 10^2$	$1,2$ ml $= 6,7 \times 10^2$	$1,4 \times 10^2$	$2,0 \times 10^1$	11	6
"	"	$1,0 \times 10^2$	$9,0 \times 10^1$	1o	6
"	"	$1,1 \times 10^2$	$7,0 \times 10^1$	14	5
$9,9 \times 10^4$	$1,2$ ml $= 1,2 \times 10^5$	$3,1 \times 10^4$	$2,0 \times 10^3$	$2,1 \times 10^3$	$9,5 \times 10^2$
"	"	$2,3 \times 10^4$	$4,3 \times 10^3$	$2,0 \times 10^3$	$5,0 \times 10^2$
"	"	$3,1 \times 10^4$	$3,0 \times 10^3$	$2,1 \times 10^3$	$11,0 \times 10^2$
"	"	$2,6 \times 10^4$	$7,8 \times 10^3$	$1,9 \times 10^3$	$10,0 \times 10^2$
"	"	$3,0 \times 10^4$	$4,3 \times 10^3$	$3,0 \times 10^3$	$8,0 \times 10^2$
"	"	$3,1 \times 10^4$	$4,3 \times 10^3$	$2,3 \times 10^3$	$7,0 \times 10^2$
$4,8 \times 10^7$	$1,2$ ml $= 5,8 \times 10^7$	$4,8 \times 10^6$	$1,0 \times 10^6$	$5,6 \times 10^5$	$3,0 \times 10^4$
"	"	$5,5 \times 10^6$	$2,1 \times 10^6$	$8,0 \times 10^5$	$4,0 \times 10^4$
"	"	$6,1 \times 10^6$	$4,6 \times 10^5$	$5,3 \times 10^5$	$3,0 \times 10^5$
$6, \times 10^8$	$1,2$ ml $= 7,2 \times 10^8$	$1,1 \times 10^8$	$1,1 \times 10^7$	$7,1 \times 10^6$	$5,3 \times 10^6$
"	"	$1,5 \times 10^8$	$1,7 \times 10^7$	$5,5 \times 10^6$	$4,3 \times 10^6$
"	"	$1,1 \times 10^8$	$3,2 \times 10^7$	$5,2 \times 10^6$	$1,8 \times 10^6$
"	"	$1,6 \times 10^8$	$1,8 \times 10^7$	$4,5 \times 10^6$	$2,0 \times 10^6$
"	"	$1,2 \times 10^8$	$2,5 \times 10^7$	$6,0 \times 10^6$	$2,2 \times 10^6$
"	"	$0,9 \times 10^8$	$1,7 \times 10^7$	$6,0 \times 10^6$	$3,0 \times 10^6$

Tabelle 4

Results with Gelatin Filter
Ergebnisse mit Gelatinefiltern
(Aerosolkammer von 6 Liter Inhalt) Chamber of 6 liters
(Versprayzeit 8 min.) Spray time 8 minutes

phage titer/ml Phagensusp.- Titer / ml	Gelatinefilter anstelle von Impinger III Gelatin filter attached to impinger III
$5,6 \times 10^2$	5×10^0
"	4×10^0
"	3×10^0
$6,7 \times 10^5$	$0,8 \times 10^4$
"	$1,0 \times 10^4$
"	$1,3 \times 10^4$
$6,0 \times 10^8$	$1,4 \times 10^7$
"	$0,7 \times 10^7$
"	$1,7 \times 10^7$
"	$1,5 \times 10^7$
"	$1,5 \times 10^7$
"	$1,3 \times 10^7$
"	$0,8 \times 10^7$
"	$0,7 \times 10^7$
"	$0,6 \times 10^7$
"	$1,1 \times 10^7$
"	$1,0 \times 10^7$

13.

Concentration of Collected phages

KONZENTRATION DER AUFGEFANGENEN PHAGEN

10^0 10^1 10^2 10^3 10^4 10^5 10^6 10^7

ABB.2

Figure 2

Concentration of starting phages

KONZENTRATION DER AUSGANGSSUSPENSION (PHAGEN PRO ML)

10^1 10^2 10^3 10^4 10^5 10^6 10^7 10^8 10^9

IMPINGER
GELATINEFILTER
MEMBRANFILTER

14.